Development of an Automated Patch Clamp Assay for recording STIM1/Orai1-mediated currents using Qube 384

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Introduction

Calcium (Ca^{2+}) is an important second messenger that is involved in critical processes in cell homeostasis and signal transduction. The ratio between extra- and intracellular Ca^{2+} contributes to setting the plasma membrane potential. Intracellularly, Ca^{2+} is stored in organelles such as mitochondria and the Endoplasmic reticulum (ER). Depletion of these stores triggers the opening of Ca^{2+} permeable channels that allow entry of Ca^{2+} into the cell along the electrochemical gradient. This process is called store-operated calcium entry (SOCE). Activation of SOCE happens as a secondary effect of ER depletion (ref). ER is being depleted by an inositol-triphosphate (IP3) pathway and phospholipase C cascade, via the IP3 receptor. SOCE can experimentally be activated by elevating intracellular levels of IP3 in the presence of a Ca^{2+} chelator.

The activation of SOCE can directly be measured as a change in clamp current when performing patch-clamp experiments. In this study the automated patch clamp system, Qube 384 was used to record I_{Crac}. Qube 384 is a second-generation automated patch clamp device capable of testing thousands of compounds per day whilst providing true gig-ohm seal quality data. Using the Qube 384 in a drug discovery cascade enables acquisition of mode of action data simultaneous with hit detection during the primary screen, thereby minimizing the need for extended follow-up validations studies.

In this study, we present an assay for STIM1/Orai1. Firstly, we present that Orai1 current can be activated either by exchange of the intracellular solution or alternative by de facto caging an agonist in the IC in the on-cell configuration. Secondly, we pharmacologically validate the assay using the trivalent lanthanide cations blocker lanthanum (La). With this, we demonstrate that Qube 384 is also a versatile patch clamp system, even though it is fully automated and can be running unattended.

Conclusion

In this study, we used the automated patch clamp set-up Qube 384, to develop an assay for measuring the current running through a calcium release-activated channel (I_{Crac}). I_{Crac} is now known to be mediated by two proteins (Orai1 and STIM1), where Orai1 is located at the cell membrane and STIM1 in the membrane of the endoplasmic reticulum (ER).

The automated patch clamp system Qube 384 operates with pre-defined plans (Figure 3 & 7), where it is easy to control whole-cell formation, protocols etc. The agonist (IP3) was added by utilizing two different methods in two separate experiments sets. Either by the exchange of the intracellular solution or by adding the agonist in the priming internal solution. Secondly, we also examined the possibilities to control the exchange using the trivalent lanthanide cations blocker lanthanum (La). With this, we demonstrate that Qube 384 is also a versatile patch clamp system, even though it is fully automated and can be running unattended.

References

1) Evidence for STIM1- and Orai1-dependent store-operated calcium influx through I_{Crac} in vascular smooth muscle cells. Role in proliferation and migration – May 2009 The FASEB Journal 23(8):2425-37